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IDENTIFICATION OF *GYMNEMA* SPECIES BY RANDOM AMPLIFIED POLYMORPHIC DNA TECHNIQUE AND CHLOROPLAST trnK GENE

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ABSTRACT

Gymnema is one of the important anti-diabetic medicinal plants used from ancient times and is commonly known as 'sugar killer'. Most of its species have been used in many applications in Indian traditional medicine. Nevertheless, their efficiency is critically dependent on the use of the correct material. The sharing of similar vernacular name and morphological features make confusion in the usage of *Gymnema* species. In the present study, *Gymnema* sp. were identified through random amplified polymorphic DNA (RAPD) technique and species specific markers were generated for easy identification of *G. elegans*, *G. montanum* and *G. sylvestre*. Using the RAPD techniques of 3 species specific markers for *G. sylvestre*, 7 markers for *G. elegans* and 4 markers for *G. montanum* had been generated. Highest genetic identity was found between *G. sylvestre* and *G. montanum* and highest genetic distance was found between *G. sylvestre* and *G. elegans*. Further, DNA barcode was developed by sequencing chloroplast partial trnK DNA of these three species. No significant variation was found in partial trnK gene sequences between *Gymnema* species. But these sequences can efficiently differentiate the *Gymnema* and *Mandevilla* species. In-silico sequence–restriction fragment length polymorphism (RFLP) analysis revealed three fragments measuring *G. sylvestre* - 204, *G. elegans* - 174, and *G. montanum* - 168 bp *Gymnema* species. The present study concluded that RAPD markers were highly efficient for species detection than the partial trnK gene sequences. This could be used to confirm the *Gymnema* sp. identities and to ensure their safe application in pharmaceuticals.

Keywords: *Gymnema* species identification, *G. sylvestre*,*G. elegans*,*G. montanum*, *RAPD*, trnK sequences, barcode

INTRODUCTION

Gymnema is an important plant genus in terms of therapeutic applications. Generally, it is referred to as sugar killer due to its anti-diabetic potential. Most of its species has been used in traditional Indian medicine for curing the disease such as diabetes, stomach ailments, constipation, water retention, and liver disease (**Chattopadhyay, 1998**). There are more than 40 species in *Gymnema* and some of them are uncategorized because of sharing of similar morphological features among the species. In that, three species of *Gymnema* namely, *G. sylvestre*, *G. elegans* and *G. montanum* have shared similar morphological traits with different medicinal properties. Notably, *G. elegans* are used as an alternate for *G. sylvestre* in traditional medicine for the treatment of diabetes (**Rana *et al.*, 1994**) and snake bite (**Masilamani, 1995**). It also possesses the antimicrobial properties. *G. montanum* is also used traditionally to treat disorders such as diabetes, high cholesterol, wounds, inflammation, and gastrointestinal ailments. Some authors have reported that extracts of some *Gymnema* species (*G. sylvestre*, *G. inodorum, and G. yunnanense*) involved in the repair mechanism or regenerate pancreatic β-cells (**Persaud *et al.*, 1999; Shimizu *et al.*, 2001; Xie *et al.*, 2003**).Likewise several *Gymnema* species shared many medicinal properties. Nevertheless, their efficiency is critically dependent on the use of the correct material. Misidentification of medicinal plants leads to severe health problems in several countries. In 2002, 63 people were reported with symptoms of general malaise, nausea and vomiting after consumption of herbal tea which was inadvertently mixed with neurotoxic Japanese star anise (*Illiciumanisatum*) (**Johanns *et al.*, 2002**). Adulteration resulting in an epidemic of severe kidney damages caused by aristolochic acid was first reported in Belgium in 1993 (**Vanherweghem *et al.*, 1993**), followed by Hong Kong and Korea (**Lo *et al.*, 2004; Lee *et al.*, 2004**). In these cases, the concerned herbs were substituted with the nephrotoxic *Aristolochia* species. A case of misusing *Daturametel* as *Rhododendron molle* was reported in Singapore in 2008 (**Phua *et al.*, 2008**). These two species share the same Chinese herb name *"Naoyanghua"*, but *D. metel* contains anti-cholinergic compound that causes confusion, dilated pupils and absence of sweating. Therefore, correct identification of medicinal plants is very important to use in therapeutic applications. Recent advancement in the molecular techniques provides several ways to examine the species. Molecular markers give information that helps in deciding the distinctiveness of species and their ranking according to the number of close relatives and phylogenetic position (**Thomas *et al.*, 2006**). Here we have applied random amplified polymorphic DNA technique and trnK gene sequences to generate species specific profile for three important medicinal plants namely *G. sylvestre*, *G. elegans*, and *G. montanum*.

MATERIAL AND METHODS

Plant material

Three species of *Gymnema* viz. *G. sylvestre*, *G. montanum* and *G. elegans* (Supplementary) were collected in the month of September from the garden of Department of Horticulture, Faculty of Agriculture, Annamalai University, Tamilnadu, India and were identified by the Taxonomist in the Department of Botany, Annamalai University. Fresh leaves were collected and kept at -80 °C for further analysis.

Genomic DNA extraction

Total genomic DNA was extracted from 100 mg of leaves using a modified protocol of CTAB (Cetyl tri-methyl ammonium bromide) (**Doyle, 1991**). A 50 µL of DNA solution was purified using ExoSAP-IT® (Affymetrix, Santa Clara, CA). The quality of purified DNA was checked using 0.8% agarose gel electrophoresis, stained with ethidium bromide and visualized under UV light. The quantity of DNA concentration was determined using UV spectrophotometer (Shimadzhu, Japan) by taking the optical density at 260 nm/ 280 nm. Final concentration of DNA was adjusted to 50 ng/ µL using double distilled water. The DNA samples were stored at -20 °C until further use.

RAPD analysis

Totally 10 plants RAPD primers were used for random amplification and their sequences are given in Tab 1. A total volume of 25 µL PCR recipe consisting 18 µL of dd.H_2O, 2.5 µL of 10X buffer with 15 mM $MgCl_2$, 2 µL of dNTP (2.5 mM each), 0.5 µL of Taq DNA polymerase (3 U/µL), one µL of RAPD primer (10 pmol/µL) and one µL of genomic DNA (1 ng/µL) was prepared out for RAPD analysis. The whole mixture was placed on a thermal cycler (TECHNE, Genei) programmed for 5 min at 95 °C followed by 40 cycles for 60 s at 95 °C, 45 s at 35 °C and 90 s at 72 °C, and finally one cycle at 72 °C for 10 min. The amplification products were resolved with 1.5% agarose gel stained with electrophoresis at 150 V and 140 mA using 1X TAE (Tris acetate with EDTA) for 3 h ethidium bromide. A 100 bp ladder was used as a molecular mass marker. The RAPD patterns were visualized in a gel documentation system (Alphaview software). For reproducibility, the concentration and the protocol was strictly followed and repeating the amplification process twice in order to score clear reproducible bands for all the ten RAPD primers. Species specific monomorphic and polymorphic bands were identified.

Table 1 Primers used for the identification of three *Gymnema* species RAPD techniques

Name of the Primers	Sequences of the Primers
RP1	5′-CAATCGCCGT - 3′
RP 2	5′-CAGAACCCAC - 3′
RP 3	5′- CTCGTGCTGG - 3′
RP 4	5′- TTCCGAACCC - 3′
RP 5	5′- GACCGCTTGT - 3′
RP 6	5′- AGGTGACCGT - 3′
RP 7	5′- TCCAACGGCT - 3′
RP 8	5′- CAGTGGGGAG - 3′
RP 9	5′- TGCGGCTGAG - 3′
RP 10	5′- ACGCACAACC - 3′

PCR amplification of the trnK region

The sequences of trnK gene primers are given in Table 2. The PCR amplification of trnK region was performed in a 25 µL PCR recipe consisting 18 µL of dd.H_2O, 2.5 µL of 10X buffer with 15 mM $MgCl_2$, 2 µL of dNTP (2.5 mM each), 0.5 µL of Taq DNA polymerase (3 U/µL), 1 µL of trnK primer (10 pmol/µL) and 1 µL of genomic DNA (1 ng/µL). The whole mixture was placed on a thermal cycler (TECHNE, Genei) programmed for 5 min at 95 °C followed by 35 cycles for 30 s at 95 °C, 1 min at 49 °C and 2 min at 68 °C, and finally one cycle at 68 °C for 10 min. The amplified products were checked with 1.5% agarose gel and stained with electrophoresis at 150 V and 140 mA using 1X TAE (Tris acetate with EDTA) for 1/2 h and ethidium bromide. The size of the amplified product was calculated using a 100 bp molecular ladder.

Table 2 Primers used in trnK gene

Name of the primers	Sequences of the primers
trnK- F	5′- GGGTTGCTAACTCAATGGTAGAG - 3′
trnK- R	5′- TGGGTTGCCCGGGGCTCGAAC - 3′

F – forward, R – reverse

Sequencing and phylogenetic analyses

The purified PCR products were sent to Macrogen, Inc. (Seoul, Korea) for unidirectional sequencing. The DNA sequence analyzer 3730xl DNA analyzer with Big Dye Terminator Cycle Sequencing Kit V3.1 (Applied Biosystems) was used for sequencing. The electropheretogram was analysed using BioEdit sequence alignment editor. Sequences with good peak clarities were selected for further analyses. The edited sequences were aligned in ClustalW program. The edited sequences were identified using BLAST program of NCBI nucleotide database. Based on the similarity index, the amplified products were assigned as trnK sequences and were recorded in the NCBI's nucleotide database with accession number JX569772, JX569773 and JX569774. Phylogenetic relationship was constructed using Neighbor-Joining statistical method. Uniform rates were followed among the sequences. Bootstrapping was performed with 1000 replications. Kimura two parameter (**Kimura, 1980**) model of evolution was followed in the analyses. All positions containing gaps and missing data were eliminated. *Mandevilla venulosa* and *M. Duartei* were used as an out group.

trnK – RFLP analysis

Restriction map of the five sequences (3 *Gymnema* species and 2 *Mandevilla* species) were generated. These sequences were online digested with *Taq*I restriction enzyme in the NEB cutter tools (New England BioLabs). Restriction map was shown in 1.4% agarose gel with 100 bp molecular ladder and the fragment sizes were calculated.

Statistical analysis

Genetic similarity/distance between the three species was estimated using PopGen Software (Version 1.31, (**Yeh *et al.*, 1999). Nei** and **Li's** (**1979**) genetic similarity (GS) among the three species was computed and converted by PopGen into genetic distance (GD) according to **Hillis** and **Mortiz's** (**1990**). Formula, GD = 1-GS. The GS reflects the proportion of the bands shared between individuals and values range from (1) when present to (0) when absent. Phylogenetic relationship was estimated based on genetic distance values generated from RAPD data among three species. A dendrogram was plotted based on **Nei** (**1978**) method using PHYLIP version 3.2 (**Felsenstein, 1993**).

RESULTS

RAPD bands

Figure 1 shows the RAPD profiles of all three species for 10 RAPD primers. All the 10 RAPD primers selected were successfully amplified and gave good RAPD profiles for the three species of *Gymnema*. Totally 427 scorable bands were observed in three species for each in the three replicates of *Gymnema*, ranging in size from 1412 to 218 bp. As observed in Tab 3, RAPD analysis revealed species specific markers like three markers viz., 787 bp by RP6, 354 bp by RP7 and 744 bp by RP10 for *G. sylvestre*, seven markers viz. 510 bp by RP3, 1078 bp, 962 bp by RP4, 825 bp by RP5, 1018 bp by RP6, 671 bp by RP7 and 378 bp by RP9 for *G. elegans* and four markers 815 bp by RP2, 923 bp by RP7, 1150 bp, 579 bp by RP8 for *G. montanum*. These species specific markers generated by these RAPD primers could be used to identify and differentiate the species of *Gymnema* unambiguously. Especially, RP7 primer could be used efficiently to discriminate the *Gymnema* species by producing species specific markers 354 bp in *G. sylvestre*, 671 bp in *G. elegans* and 923 bp in *G. montanum* and in addition these bands can be clearly figured out in the agarose gel electrophoresis. Much difference was not observed in the frequencies of polymorphic bands between the three species. The polymorphic percentage of *G. sylvestre*, *G. elegans*, and *G. montanum* were predicted as 38.32%, 39.56%, and 40.81%, respectively.

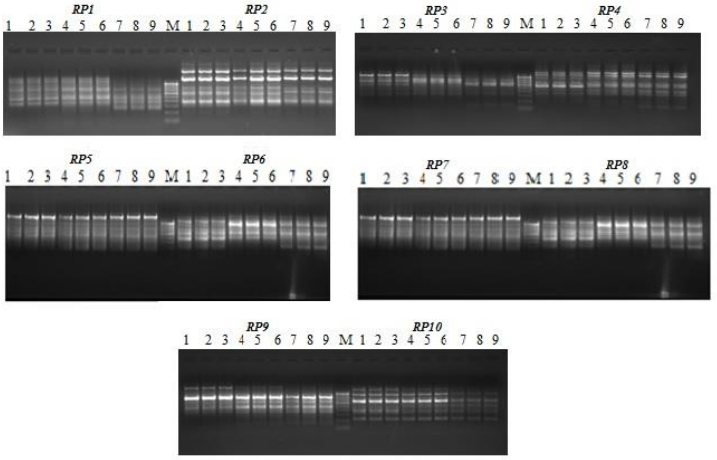

Figure 1 RAPD banding pattern of *Gymnema* species using the primers *RP1* to *RP10* (Lanes: 1-3 *G. sylvestre*; 4-6 *G. elegans;* 7-9 *G. montanum*. M – 100 bp marker ladder)

Table 3 Number of monomorphic and polymorphic bands in three species of *Gymnema* by RAPD

	Gymnema sylvestre			*Gymnema elegans*			*Gymnema montanum*		
Name of the primer	RAPD band range (bp)	No.of monomorphic bands (size)	No.of polymorphic bands	RAPD band range (bp)	No.of monomorphic bands /size (bp)	No.of polymorphic bands	RAPD band range (bp)	No.of monomorphic bands /size	No.of polymorphic bands
RP1	1355-480	-	18	1327-610	-	18	1418-780	-	22
RP2	1136-150	-	14	1136-290	-	17	815-420	1 (815)	10
RP3	1320-250	-	16	1320-218	1 (510)	14	1320-218	-	20
RP4	1078-262	-	10	1078-310	2 (1078,962)	11	928-300	-	12
RP5	1211-362	-	14	1078-367	1 (825)	14	1044-423	-	18
RP6	1155-253	1(787)	12	1155-251	1(1018)	14	1155-305	-	9
RP7	993-260	1 (354)	15	1091-245	1 (671)	14	923-258	1 (923)	9
RP8	1090-310	-	12	1110-265	-	18	1150-270	2 (1150,579)	16
RP9	1145-365	-	18	1127-363	1 (378)	13	1164-363	-	8
RP10	1150-338	1 (744)	14	1158-320	-	12	938-384	-	13

The genetic identity and genetic distance between the species of *Gymnema* is given in Tab 4. The genetic identity (above diagonal) ranged from 0.8968 to 0.9153 and inter-species distance ranged from 0.0885 to 0.1089 based on Nei's band sharing frequencies. High genetic identity was found between *G. sylvestre* and *G. montanum* and highest genetic distance was found between *G. sylvestre* and *G. elegans*.

Table 4 Nei's genetic identity (above diagonal) and genetic distance (below diagonal) of *Gymnema* species.

Species	***G. sylvestre***	***G. montanum***	***G. elegans***
G. sylvestre	****	0.9153	0.8968
G. montanum	0.0885	****	0.9035
G. elegans	0.1089	0.1014	****

Based on genetic distances, dendrogram was generated by UPGMA method which showed the relationship between the *Gymnema* species. Figure 2 depicts the dendrogram of *Gymnema* with bootstrap support value. *G. sylvestre* and *G. montanum* exhibits close relationship while *G. elegans* occurred as a single main clade.

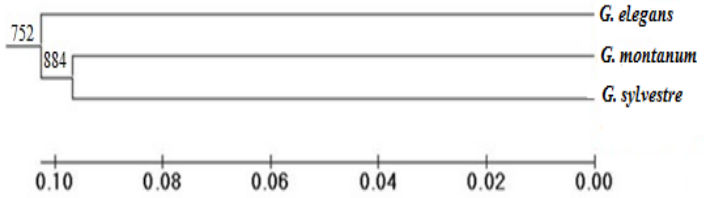

Figure 2 UPGMA dendrogram using Nei's genetic distances inferred from RAPD (Bootstrap values based on 1000 replications given in the nodes)

trnK

trnK-F and trnK-R primers were successfully amplified using *Gymnema* species and the amplified products were predicted to have 700 bp in length (Figure 3).

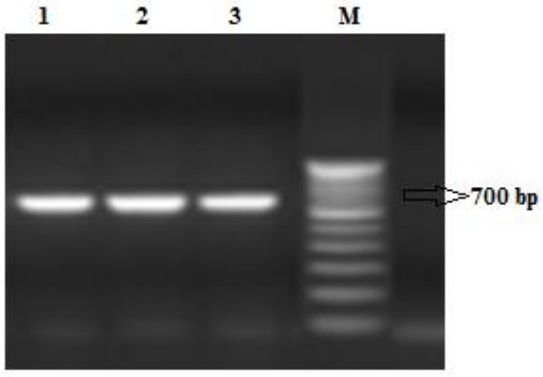

Figure 3 trnK gene sequence amplified by polymerase chain reaction (Lanes: 1 – *G. sylvestre*; 2 – *G. elegans*; 3- *G. montanum*; M – 100 bp plus DNA ladder)

The trnK gene sequences of the three *Gymnema* species were analysed by dideoxy sequencing method and found to have 549, 653, and 573 bp length, respectively. The sequences were short of matK region at 3´ end of the trnK sequence. The trnK sequences of *G. elegans* and *G. montanum* were identical. *G. sylvestre* showed three distinct insertion of adenine nucleotide at 446, 524, and 541 positions at 3´ end. Remaining sequences were identical with *G. elegans* and *G. montanum.* On Blast analysis, these sequences exhibited 94% identity (100% query coverage) with trnK sequences of *Mandevilla* species as both *Mandevilla* and *Gymnema* belong to Gentianales order. These sequences were barcoded and deposited as JX569772, JX569773 and JX569774 in NCBI's nucleotide database for identification.

The phylogenetic tree of trnK sequence showed two major clades one for *Gymnema* and another *Mandevilla*. The *Gymnema* clade showed no distinct sub clade pattern, and no differences in the distance scale among the three species and with 100 % bootstrap support (Figure 4).

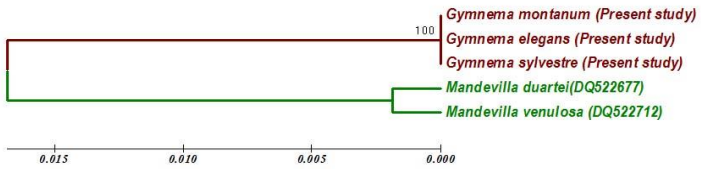

Figure 4 UPGMA Phylogenetic tree from partial trnK gene sequences of *Gymnema* and *Mandevilla* species (Bootstrap value (%) is shown on branch and the scale represents nucleotide substitution per site)

The trnK gene sequence of *Gymnema* species by RFLP showed no specific restriction pattern after digestion with *Taq*I enzyme. Each *Gymnema* species showed three fragments measuring 204, 174, and 168 bp. The out group *Mandevilla* species showed two fragments measuring 331 and 204 bp (Figure 5).

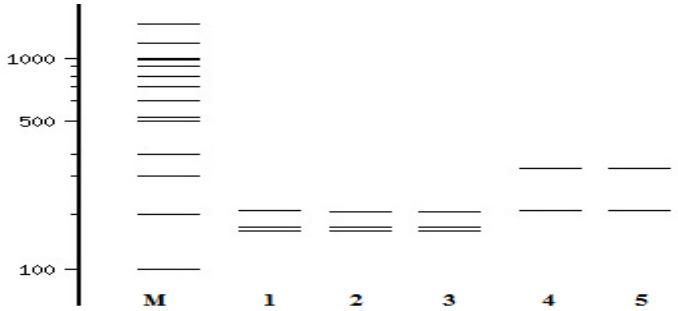

Figure 5 RFLP profiles generated from trnK sequences of three *Gymnema* and two *Mandevilla* species after digestion with *Taq*I restriction enzyme (Lanes: 1 – *G. sylvestre*; 2 – *G. elegans*; 3- *G. montanum*; 4- *M. duartei*; 5- *M. venulosa*; M – 100 bp plus DNA ladder)

DISCUSSION

The medicinal herbs *G. sylvestre*, *G. elegans*, and *G. montanum* are called by the same vernacular name in Tamilnadu as 'Chakkarakolli'. However, these species exhibits different efficacy and used in different applications (**Chattopadhyay, 1998; Wiriyakarun and Yodpetch, 2013**). This type of name sharing makes

confusion on choosing herbal plants and resulting in medication errors that has been reported in many countries (**Nadkarni, 1992**). According to WHO guidelines on research and evaluation of traditional medicine, first step is assuring quality, safety and efficacy of traditional medicine for correct identification. Hence, the present study attempted to develop species specific markers using RAPD markers and DNA barcode using a trnK gene sequences.

Species identification by RAPD

RAPD is a quick and cost-effective molecular technique generating anonymous loci that are randomly scattered in the genome (**Clark and Lanigan, 1993**). This technique is one of the most frequently used molecular methods for taxonomic and systematic analyses of various organisms (**Garg *et al.*, 2009**). RAPD has been successfully utilized for the identification and genotyping of medicinal plants (**Tochika-Komatsu *et al.*, 2001**; **Um *et al.*, 2001**), herbal medicinal components (**Shinde *et al.*, 2007**), Ornamental plants (**De Benedetti *et al.*, 2001**) and other varieties of plant species (**Temiesak *et al.*, 1993**). This technique can able to differentiate the taxa below the species level (**Choo*et al.*, 2009**), because RAPD analysis performed both coding and non-coding regions (**Vanijajiva *et al.*, 2005**).The reproducibility of the RAPD markers highly depends upon the quality, quantity of the template DNA and standard PCR conditions (**Dinesh *et al.*, 1995**). In the present analysis, the concentration of the DNA, concentration of PCR recipes, cycling program, and electrophoretic conditions were consistently maintained for all the 10 RAPD primers and therefore, the markers obtained in the present study are considered to be highly reproducible. These markers can be used for identification of polymorphic status and species identification of *Gymnema* in future.

The polymorphic percentage of *G. sylvestre* was found to be 38.32%. This was very low when compared to two previous reports (**Smita and Keshavachandran, 2006; Balamurali *et al*., 2012**), where different forms of *G. sylvestre* have been collected from different regions of India and showed 73.2% and 50% polymorphism. Relatively low level of genetic polymorphism in this study is due to the small sampling size. Moreover, RAPD primers used in this study revealed notable polymorphism among the *Gymnema* species. Similarly, considerable polymorphism was found in closely related medicinal herbs *Curcuma* sp. in Yakushima with *C. aeruginosa* and *C. zedoaria* in Java by RAPD markers (**Agusta *et al.*, 2007**).

The species specific bands obtained in the present study ranged from 354 to 1150 bp. The species specific markers obtained 354 bp in *G. sylvestre*, 671 bp in *G. elegans* and 923 bp in *G. montanum* by RP7 primer could be used for species identification in *Gymnema* species. Similarly unique bands were obtained in medicinal plant *Sennaangustifolia* species by using different RAPD primers that were clearly discriminate the species (**Salim *et al.*, 2011**) . In addition, geographical specific bands ranged from 130 to 2340 bp were observed in medicinal plants in the previous studies (**Smita and Keshavachandran, 2006**). Hence, the species specific markers developed in the present study could be used in the identification of these *Gymnema* species. The UPGMA dendrogram based on Nei's genetic distance revealed the relationship among the three species of *Gymnema*. The *G. sylvestre* and *G. montanum* formed a single cluster indicating that sharing of little RAPD banding pattern between these two species (Figure 2).

Species identification by trnK gene sequence

The feasibility of chloroplast trnK gene sequence as a DNA barcode of the *Gymnema* species was concretely tested. The partial trnK sequencing showed advantages of higher efficiency of PCR amplification and sequencing. However, the partial trnK sequencing is insufficient to discriminate the *Gymnema* species, as the identification efficiency was very low. Multiple sequence alignment showed that *G. sylvestre* had only few numbers of insertion sites in the trnK gene sequences. The sequences were highly conserved and no variation was found. In the BLAST analysis, all the three sequences showed maximum identity with *Mandevilla* genus. Hence, *Mandevilla* species was selected as an out group for phylogenetic tree construction and both *Mandevilla* and *Gymnema* belonged to the same order *Gentianales*. Similarly, in the previous studies (**Efron *et al.*, 1996**) several species have similar sequences with no variable sites in the trnK gene sequences. For example, even though *Elettarriopsis* and *Paramomum* were categorized under unique taxonomic position by morphological characters of flowers, they shared similar features and highly conserved with 100% identity with no variant sites in the trnK sequences (**Dhivya *et al.*, 2008**).

The present study showed that species identification of *Gymnema* herb was not possible using phylogenetic analyses constructed from partial trnK sequences. *Gymnema* and *Mandevilla* species (out group) formed two separate clades. The *Gymnema* species formed a single clade suggesting that these species evolved from a common ancestor. And *Mandevilla* species also showed a single clade. This clade pattern simply reflects the sequence similarity between the species. But in the previous study, this region had enough diversity to discriminate the *Curcuma* sp. (**Agusta *et al.*, 2007**). Noticeably, it was found that these trnK gene sequences were used as most useful barcode and provided a universal framework for land plants at and above the generic levels (**Ren and Chen, 2010**).

Several authors have used the following DNA region for generation of DNA barcode *rbcL*, 18s rRNA, *psbAtrnH*, and ITS2 intergenic spacer. Depending upon the plant species and evolutionary rate each region has different efficiency in species identification. Higher efficiency of species discrimination was found in trnK gene than the *rbcL* gene in *Dendrobium* species (**Asahina and Shinozaki, 2010**). It was reported that (**Liu *et al.*, 2012**) *psbA-trnH* intergenic region is a potential DNA barcoding sequence for identifying the *Rhododendron* species. **Sun *et al.*** (**2012**) concluded that *trn*K is a strong, although not perfect, candidate as a DNA barcode for *Dioscorea* identification. From the analysis of previous findings and our results we conclude that 5′ partial trnK gene sequences of *Gymnema* species not sufficiently discriminate at species level. Other DNA regions (*rbcL*, *psbAtrnH*, and ITS2 intergenic spacer) should be analyzed to find the best DNA region as a DNA barcode for identification of *Gymnema* species.

PCR-RFLP has been used in several studies and successfully authenticated the medicinal plants such as *Mitragyna speciosa*, *Phyllanthus* species, *P. amarus, P. debilis, P. urinaria,Pueraria candollei, Butea superb* and *Mucuna collettii* (**Quinteiro *et al.*, 2001; Manissorn *et al.*, 2010; Wiriyakarun and Yodpetch, 2013**). In this regard, we attempted to generate a restriction pattern of *Gymnema* and *Mandevilla* with in-silico trnK sequence - RFLP analysis. There was no specific restriction pattern observed within the *Gymnema* or *Mandevilla* species but different restriction patterns were observed between the *Gymnema* and *Mandevilla* species. These restriction patterns will be reference from PCR-RFLP analysis for these species and no need to going for sequencing.

CONCLUSION

The correct identification of medicinal plants is vital for their conservation and sustainable use, as well as to prevent fake in the marketing of medicinal plants. RAPD and DNA sequence based identification system were used in several plant species (**Um *et al.*, 2001; Ren and Chen, 2010**). The RAPD primers used in the present study developed species specific markers which could be used for identification of *G. sylvestre, G. elegans* and *G. montanum* unambiguously and trnK gene sequences for three species could not be used for identification of species. Present study concluded that the RAPD markers are a candidate for *Gymnema* species identification than the partial trnK gene sequences.

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SUPPLEMENTARY PART

Figure 1 *Gymnema sylvestre*

Figure 2 *Gymnema elegans*

Figure 3 *Gymnema*